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## STUDIES ON THE PLANT CELL.—III.

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### SECTION III. HIGHLY SPECIALIZED PLANT CELLS AND THEIR PECULIARITIES.

VERY much of our knowledge of the structure and behavior of protoplasm in plants has been derived from the study of certain cells whose organization has reached an exceptionally advanced degree of differentiation. The peculiarities of these cells are obvious and have proved of great interest but we have as yet scarcely made a beginning in the study which must trace and relate these characteristics of the most complex products of cellular evolution in plants to their more simple progenitors.

This section will describe in some detail the structure and protoplasmic activities of the following six highly specialized cells: 1, The Zoöspore; 2, The Sperm; 3, The Egg; 4, The Spore Mother-Cell; 5, The Cœnocyte; 6, The Cœnogamete.

#### 1. The Zoöspore.

Zoöspores are interesting not only for their own peculiarities but also because they are well known to be the progenitors of the sexual cells or gametes which become later differentiated into the egg and sperm. Comparative studies upon three cells so closely related and yet so diverse in their extremes of structure are sure to yield important results.

The zoöspore is generally an uninucleate cell, colorless in the Fungi, but containing a chromatophore or plastids in all other groups of thallophytes. There are usually two or four cilia attached to the anterior pointed end which is free from coloring matter and at this region one may expect to find a red pigment spot. Some zoöspores are exceptional for special peculiarities, as those of *Vaucheria* which are multinucleate, each nucleus

being accompanied by a pair of cilia, or those of *Ædogonium* whose colorless forward end bears a crown of numerous cilia. The zoöspore stands among the higher forms for a type of motile organism that is very close to the bottom of the assemblage of groups and developmental lines which make up the Algæ. The forms most closely related to the zoöspore are in the family Chlamydomonadeæ of the Volvocales. But at this general low level of the plant kingdom there are several groups whose members pass most of their lives in motile conditions (Volvocales, Flagellates and Peridinales) and the cells of all of these types resemble zoöspores to a greater or less degree in their structure and habits, so that this condition represents a widespread and well defined stage of evolutionary development. Therefore when zoöspores are formed in the life history of some higher plant they represent a return on the part of the organism for a short time to the structure and mode of life of an ancestry perhaps related in some way to the groups that still have the motile habits throughout most of their existence.

For these reasons close comparisons in structure between the zoöspore and motile Algæ will be interesting and should help to explain the peculiarities of these cells. These peculiarities chiefly concern the organ that forms the cilia (blepharoplast), which becomes very complex in the sperm, and the pigment spot.

Unfortunately studies upon these problems have been few and we are not prepared to make a general statement of the conditions. The most recent investigation on the structure of the zoöspore is that of Timberlake (:02), but Strasburger has written extensively on the subject, especially in the *Histologische Beiträge* ('92 and :00). The later paper (:00, p. 177-215) reviews the entire subject of cilia formation. Dangeard has presented an account of the Chlamydomonadeæ, '99, and in :01 described especially *Polytoma*, comparing its structure with that of the animal spermatozoan.

*Polytoma* (see Fig. 9 a) is a colorless organism but its cell structure and life history place it unquestionably among the Chlamydomonadeæ. The two cilia arise from a small body (blepharoplast) situated at the extremity of the cell. A delicate

thread-like structure, which Dangeard calls the rhizoplast, extends from the blepharoplast into the cytoplasm and sometimes ends at the side of the nucleus in a granule (condyle). The cilia grow out from the blepharoplast. This apparatus is not known to bear any relation to centrosomes or to the kinoplasm of nuclear figures present at the time of spore formation. But it should be noted that the blepharoplast is situated directly under if not actually in the outer plasma membrane, which is kinoplasmic. The filamentous connection between blepharoplast and nucleus is probably important, especially since it has also been found in zoöspores (Timberlake, :02, for *Hydrodictyon*) but we do not even know its developmental history much less its function. Further study will be necessary to make clear possible relations to kinoplasm around the nucleus or to centrosomes. Consequently Dangeard's comparison of *Polytoma* to the animal spermatozoön is not convincing for it seems to be established for the spermatozoön that portions of the middle piece at least and the flagellum are derived from a true centrosome. Indeed from the meager evidence now at hand the blepharoplast of *Polytoma* is as likely to be a structure differentiated from the plasma membrane as to have any relation to the nucleus. But detailed studies on sporogenesis may discover a history more in harmony with that of *Hydrodictyon*.

We have summarized a portion of Timberlake's (:02) account of sporogenesis for *Hydrodictyon* in the previous section under the head of "Cleavage by constriction." We shall consider now certain details. Small spherical bodies are found at the poles of the spindles during nuclear division in the mother-cell. They are undoubtedly accumulations of kinoplasm and perhaps stand for centrosomes. However they have no polar radiations nor could they be followed between mitoses when the nuclei were in resting conditions. It is not probable therefore

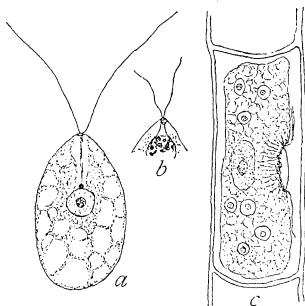


FIG. 9.—The Zoöspore. *a*, *Polytoma*; *b*, *Hydrodictyon*; *c*, Development in *Oedogonium*. (*a*, after Dangeard :01; *b*, Timberlake :02; *c*, Strasburger '92.)

that these structures are permanent in the cell. After nuclear multiplication is ended segmentation proceeds until the nucleate masses of protoplasm separate from one another as zoöspores. Then a body may be found lying in contact with the plasma membrane and bearing a pair of cilia (Fig. 9*b*). This basal body (blepharoplast) by its reaction to stains seems to be entirely distinct from the plasma membrane and is connected with the nucleus by very delicate threads. There is a time just previous to the differentiation of the zoöspores when the nuclei lie very close to the cleavage furrow that finally separates the adjacent zoöspore origins. A granule may sometimes be observed close to these nuclei and it is possible that this is the first appearance of the basal body (blepharoplast). If this should prove correct the structure may have a direct relation to the kinoplasm around the nucleus, a relation that is afterwards maintained through the two or three delicate fibers that connect these structures. Thus the blepharoplast if not directly derived from a centrosome may at least have its origin from the same region of kinoplasm. However these possibilities are mere speculations and the investigation of these points is very much to be desired in a number of algal and fungal types.

We are now brought to the views of Strasburger as expressed in his writings of '92 and :00. His investigations have been chiefly on *Vaucheria*, *Cladophora* and *Ædogonium*. In all of these forms the cilia come from a body (blepharoplast) which he believes to arise from the outer plasma membrane (*Hautschicht*). The nucleus lies close to the plasma membrane at the time when the blepharoplast is formed and may determine its development there as a dynamic center, but the blepharoplast is not a centrosome according to Strasburger. It is of course kinoplasmic since it develops from the plasma membrane and this would accord with its activities as a cilia forming organ. The blepharoplast is extraordinarily large in *Ædogonium* (see Fig. 9*c*) and develops a ring of numerous cilia on the exterior while at the same time fibrillar rays grow back into the cytoplasm and probably help to give a compact organization to the zoöspore. This structure is very suggestive of the centrosphere and aster that cuts out the ascospore (see Section II, Free Cell

Formation) and in spite of Strasburger's conclusions that it is derived entirely from the plasma membrane we are justified in asking for a fuller description of its development. There is the possibility of a different origin wherein the nucleus may play an important part which, in the light of Timberlake's studies on *Hydrodictyon*, suggests that Strasburger may not have discovered the earliest beginning of the blepharoplast in *Ædogonium*. And the same doubts apply to *Cladophora* and *Vaucheria*.

There is thus considerable divergence in the views of the origin and nature of the blepharoplast in zoöspores, Strasburger believing that they are developed as a specialized region of the plasma membrane with no relation to centrosomes, and Timberlake holding that the structure in *Hydrodictyon* is not a part of the plasma membrane but comes from the interior of the protoplasm. The problem is also involved with conditions in the sperm, where there is likewise a difference of opinion as to the homologies of the blepharoplast but an undoubted origin at least in the pteridophytes and gymnosperms from the interior of the cell. We should naturally expect the blepharoplasts of zoöspores and sperms to be homologous and consequently the problem is of great theoretical interest and will be taken up again in our discussion of the sperm. Its solution demands a most thorough study of the development of some of the larger zoöspores as in *Ædogonium* and certain species of the *Conferales* and *Volvocales*.

The pigment spot is almost universally present in zoöspores and is also characteristic of the cells of many motile organisms as in the *Volvocales* and *Flagellates* while occasionally found in other groups. The structure has been called an eye spot from its fancied resemblance to the simple eyes of certain *Crustacea* (*Cyclops*, etc.) but this term is unsatisfactory since it is not established that the pigment spot is primarily a receptive organ for light or warmth; but even should it prove to be thus sensitive (which is very probable) thereby orienting the cell with respect to the direction of incoming rays, that is not a function comparable to sight.

The coloring matter of the pigment spot is held as a single globule or as a collection of numerous small granules in meshes

of the protoplasm. It is frequently associated with a plastid. The pigment may be readily broken down and dissolved out by such reagents as alcohol and ether. In chemical composition it is very close to hæmatochrome and thus may be related to chlorophyll or a derivative of that substance. The cytoplasm around the pigment spot is undifferentiated and when the coloring matter is removed it is very difficult and sometimes impossible to find the situation of the structure. Consequently the pigment spot can hardly be considered a protoplasmic organ since it is merely an accumulation of coloring matter at some point in the cell. Strasburger (:00, p. 193) states that the pigment spot of certain zoöspores (*Cladophora*, etc.) is formed in the plasma membrane but this is not true of many other motile cells (*Flagellata*) and there is no doubt that in some cells (*e. g.* the gametes of *Cutleria*) the pigment spot is a portion of a plastid. The literature upon the structure and function of pigment spots is reviewed by Zimmermann (*Beiträge z. bot. Centralb.* Bd. 4, p. 159, 1894) and since then Wager ('99) has presented a detailed study of *Euglena*.

## 2. The Sperm.

The sperm is unquestionably derived from the zoöspore through primitive types of gametes which were identical with zoöspores in all essentials of morphology. I have described the origin and evolution of sexual cells of plants in two recent papers (*Popular Science Monthly*, Nov. 1901, p. 66 and Feb. 1902, p. 300). We should expect the simplest forms of sperms to have the characters of zoöspores and this is the fact. The sperms of the Algæ, as a rule, have the same number of cilia (usually two) as their ancestral asexual zoöspores. They generally contain a chromatophore, although sometimes much reduced, and there is present the pigment spot. The cilia are attached at the pointed end or at the side, arising from colorless protoplasm that sometimes contains the pigment spot while the chromatophore, when present, and the nucleus lie at some distance from this region of the cell. The sperms of bryophytes and pteridophytes are much attenuated in form and lack the

pigment spot and chromatophore. Those of the bryophytes and the Lycopodineæ are biciliate while other pteridophytes have multiciliate sperms the cilia being distributed on a band (blepharoplast) which lies along one side of the spiral structure. A large portion of the spiral in these sperms is composed of nuclear substance and much of the remaining cytoplasm with granules and vacuolar inclusions may frequently be found in a vesicle attached to the larger end of the spiral.

The only motile sperm among the Fungi is that of *Monoblepharis*. The male cells of other Fungi are non-motile bodies (spermatia) generally formed from the ends of delicate filaments which are found in special organs called spermatogonia. Spermatogonia have been described in the Uredinales, the lichens and in the Laboulbeniaceæ but their function is only clearly established for the last two groups. They are very highly differentiated in the Laboulbeniaceæ and comprise several types of structure. Another type of male cell, found in certain groups of the Phycomycetes and Ascomycetes, is the cœnogamete (to be described presently) which is however not the homologue of the sperm but of the mother-cell or antheridium that develops such structures. Sperms of the red Algæ (Rhodophyceæ) are likewise non-motile and they are invariably formed singly in small cells at the ends of filaments. These non motile sperms of Fungi and red Algæ are exceedingly small uninucleate bodies without further complexity of structure as far as is known.

We shall not attempt to discuss the earlier literature that treats of the structure and development of the plant sperm. In 1894 Belajeff published a German translation of a paper written two years before in Russian which presents the views of previous investigators and to this the reader is referred for such historical references. At that time various opinions were held respecting the organization of the sperm, some writers (Campbell, Guignard and others) believing that it was chiefly or wholly nuclear in origin, while another group (Zacharias, '87, Belajeff, Strasburger, '92, etc.) thought that the cytoplasm shared very largely in its structure. Belajeff ('94a) from studies among the Characeæ showed with especial clearness that the cytoplasm was an important constituent of this sperm since the nuclear



material occupied a restricted region in the middle of the spiral structure. This was the first of a series of investigations which have given especial attention to cytoplasmic activities during spermatogenesis and placed the entire subject in a new light.

The year 1897 brought forth almost simultaneously three short papers by Webber ('97a, '97b, '97c) and Belajeff ('97a, '97b, '97c) respectively. Webber had studied the development of the motile sperms of *Zamia* and *Ginko*, Belajeff certain forms of the *Filicineæ* and *Equisetineæ*. These were of the nature of preliminary announcements and both authors published later more detailed descriptions and discussions. The discoveries of motile sperms in *Ginko* by Hirase and of *Cycas* by Ikeno were announced in several short papers during the years 1896 and '97 but without descriptions of their development. This literature together with later papers of Ikeno, Shaw, Belajeff, Hirase, and Fujii is reviewed in Webber's last contribution (:01) and also in Strasburger's discussion of "*Cilienbildner*" (:00, p. 177) to which the reader is referred for the most complete treatments of spermatogenesis in plants yet published.

The cycads and *Ginko* are the most favorable subjects known for studies in spermatogenesis. Detailed accounts of the cycads are given by Ikeno ('98b) for *Cycas* and by Webber (:01) for *Zamia*, these forms agreeing with one another in all essentials. Two sperms are developed from the daughter cells (spermatids) following the division of the so-called body cell in the pollen tube. The process really begins in the body cell with the appearance of the blepharoplasts. Their development has been followed with especial attention in *Zamia*. They are formed *de novo* in the cytoplasm at some distance from the nucleus and while the latter is in the resting condition. They appear independently of one another, generally on opposite sides of the nucleus but sometimes much nearer together (Fig. 10 *a*). Each is a large deeply staining body with numerous radiations extending into the cytoplasm. The blepharoplasts then increase in size and, moving farther away from the nucleus, take positions exactly opposite to one another. The nucleus of the body cell now divides, its spindle being clearly intranuclear (Fig. 5 *d*) and consequently holding no visible relation to the blepharoplasts

which lie at a considerable distance from the structure (Fig. 10 *b*). The latter cannot then be said to occupy the position of centrosomes in relation to this spindle. Meanwhile important changes, which are best known for *Zamia*, take place in the blepharoplast. In this type the structure forms a hollow sphere which breaks up into segments and finally into granules as mitosis proceeds. The radiations disappear without holding any apparent relation to the spindle. During telophase each of the two blepharoplasts

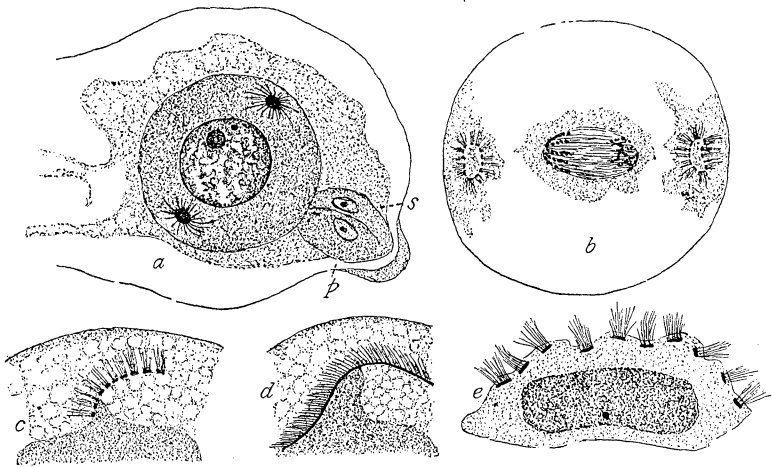


FIG. 10.—Spermatogenesis in *Cycas*. *a*, Body cell in pollen tube with two blepharoplasts; *s*, stalk cell; *p*, prothallial cell; *b*, anaphase of mitosis in the body cell the spindle lying between the two blepharoplasts which have begun to form cilia; *c*, Blepharoplast elongating, in contact with a process from the nucleus; *d*, end of blepharoplast attached to the nucleus at a later stage of development; *e*, sperm showing section of the flattened spiral blepharoplast with cilia projecting beyond the cell. (After Ikeno, '98.)

appears as a mass of granules at some distance from the daughter nuclei which are to become the sperm nuclei. As a result of this division the spermatids (sperm mother-cells) are differentiated. At the close of the mitosis the blepharoplast enters upon its functions of forming in the spermatid a cilia bearing band which is to lie as a spiral around the sperm. The granules first extend as a delicate deeply stained line towards the nucleus and then in the opposite direction. The nucleus in *Cycas* puts forth a papilla (Fig. 10 *c*) which meets this line of granules and remains attached to it for some time. The line thickens into a

band which lengthens and finally takes the form of a spiral of five or six turns which becomes more or less closely applied to the plasma membrane (Fig. 10 *c*, blepharoplast in section). The cilia develop as protuberances from the outer surface of the band (Fig. 10 *c* and *d*) and grow through the plasma membrane to the exterior of the cell. The nucleus in the meantime has increased in size until it occupies the greater part of the top shaped sperm (Fig. 10 *c*).

The history of spermatogenesis in Ginko is strikingly parallel to that of the cycads. The chief features were first described by Webber ('97c) and in greater detail by Hirase ('98). The two blepharoplasts appear *de novo* on opposite sides of the nucleus in the body cell. They show the same high state of differentiation as those of the cycads, being large and the center of a number of prominent radiations. Ginko however presents a peculiarity not reported in the previous group. A large spherical body lies between each blepharoplast and the nucleus in an area of granular cytoplasm. This structure stains deeply like the globules of nucleolar substance which are frequently found in the cytoplasm after nuclear division. They are probably accumulations of a somewhat similar material at these points in the cell to be utilized at later periods of spermatogenesis, since they decrease in size as the sperms mature. The spindle in the body cell is formed between the blepharoplasts but its poles lie at some distance from and are entirely independent of these structures. During this mitosis the spherical bodies pass to one side of the spindle so that the daughter nuclei (sperm nuclei) finally take the position formerly occupied by them. The blepharoplast becomes granular and begins to lengthen into a band, one end of which becomes attached to the nucleus that puts forth a small papilla towards the blepharoplast. The band elongates and takes the form of a spiral which makes several turns around one end of the cell just under the plasma membrane. Cilia then develop along this band as in the cycads. The earlier accounts, describing a short tail on the sperm were founded upon material that was not altogether normal and have been corrected by Webber and Fujii. The mature sperms have essentially the same form as those of *Zamia* and *Cycas*.

There has been some discussion on the morphology of these motile sperms of the gymnosperms. The claim has been made that they are ciliated spermatids (sperm mother-cells) and therefore different from the sperms of pteridophytes which are formed inside of mother-cells that upon their escape are left behind as empty cysts. However a close analysis of their structure will show that the sperms in both groups have an identical protoplasmic organization. There is a nucleus and a greater or less amount of cytoplasm in which the blepharoplast lies and the entire structure is surrounded by a plasma membrane. Any differences in the processes of spermatogenesis can only concern the greater or less development of a cellulose membrane around the spermatids. It may be true that this cellulose membrane is entirely absent in *Cycas* and *Zamia*, but if present it would be merely a shell like envelope around the sperm and cannot affect its morphological unity and agreement with the sperms of pteridophytes. A comparative study of the composition and formation of the walls enclosing sperm nuclei in the spermatophytes is much needed to carefully distinguish between plasma membranes and the cellulose secretions that may be developed by them.

While the cycads and Ginko have very much the largest sperms known and are consequently extremely favorable for an examination of spermatogenesis nevertheless some surprisingly detailed studies have been made among the Filicineæ and Equisetineæ. Following his preliminary announcements ('97a, '97b, '97c), Belajeff published in '98 an account of spermatogenesis in *Gymnogramme* and *Equisetum*. These forms present histories parallel to each other and to the cycads. Two deeply staining bodies (blepharoplasts) appear on opposite sides of each nucleus previous to the final mitosis in the antheridium which differentiates the spermatids. Consequently each spermatid receives a blepharoplast which lies close beside the nucleus. The blepharoplast begins to elongate and is followed by the nucleus so that both structures form two parallel bands which take a spiral form. (Illustrated in Fig. 3½ of Section I.) The rest of the cytoplasm remains as a vesicle which comes to lie at the larger end of the sperm. The cilia of *Equisetum* could be traced to

definite granules in the band as it develops from the compact spherical blepharoplast.

There appeared almost simultaneously with the foregoing contribution of Belajeff a paper by Shaw ('98b) on *Onoclea* and *Marsilia*. Shaw investigated the cell divisions preceding the formation of the spermatids in *Marsilia* and discovered some very interesting conditions. The two blepharoplasts which are found in the mother cell of the spermatid are foreshadowed by smaller bodies which appear at the poles of the spindle in the two previous mitoses. The first of these structures was called a blepharoplastoid. The blepharoplastoid first appears besides the daughter nucleus after the third mitosis previous to the differentiation of the spermatids. There is therefore one for each nucleus of the grandmother cell of the spermatid. This blepharoplastoid divides but the halves remain close together and the pair passes to one side of the cell. With the next mitosis (the second previous to the differentiation of the spermatids) two new structures are formed at the poles of the spindle and from these the blepharoplasts arise. They accompany each daughter nucleus after this mitosis into the mother-cell of the spermatid. Then each divides and the two blepharoplasts pass to opposite sides of the nucleus which prepares for the final mitosis of the series. This division gives a daughter nucleus to each blepharoplast and the spermatid is thus organized. The later history of the spermatid as it changes into the sperm is identical with Belajeff's results.

Belajeff ('99) followed Shaw's account of *Marsilia* with a study of the same form and came to very different conclusions which have to do chiefly with his belief that the blepharoplast is a centrosome, a view that will presently be considered in connection with the opinions of Strasburger and others. Belajeff found centrosome like bodies (blepharoplastoids of Shaw) at the poles of spindles in various mitoses preceding the formation of the spermatids with their unquestioned blepharoplasts. He is not willing to concede that these centrosome like structures pass into the cytoplasm to disappear there as Shaw states for the blepharoplastoids. He also found the blepharoplasts at the poles of the spindles, which was not observed by Shaw, and holds that they have a part in spindle formation.

We are now prepared to take a general survey of the processes of spermatogenesis to harmonize as much as possible the conflicting opinions respecting the homologies of the blepharoplast. Strasburger (:00, pp. 177-215) has critically reviewed the subject and his conclusions are of great interest. He emphasizes the kinoplasmic character of the blepharoplast, whether it be a differentiated region of the plasma membrane (as he believes for the zoöspores of *Cladophora*, *Cedogonium*, etc.) or a special development in the interior of the cytoplasm (pteridophytes and gymnosperms). Strasburger thinks that all kinoplasmic structures, be they centrospheres, centrosomes or blepharoplasts, hold a very close physiological relation to the substance of the nucleolus and that their appearance and size is largely the result of nuclear activities. Accordingly the blepharoplast might occupy the position of a centrosome without being genetically related to that structure, and in fact centrosomes or centrospheres are to be considered more as products of the cells' activities than as self perpetuating permanent organs. There is abundant evidence that the last possibility is the fact in many forms both plants and animals. Since centrosomes are not found at other periods of the life history of gymnosperms and pteridophytes, Strasburger concludes that the blepharoplasts cannot be genetically related (homologous) with such a structure.

Ikeno and Hirase from their earliest writings have considered the blepharoplast to be a centrosome. Ikeno ('98a) held that the blepharoplast corresponded with the middle piece of the animal spermatozoon. Hirase ('94 and '97) although noting for *Ginkgo* that the blepharoplasts did not divide and took no part in spindle formation nevertheless called them attractive spheres. The conclusions of Shaw ('98) and Belajeff ('99) for the same type (*Marsilia*) have just been summarized and present very different points of view. Belajeff believes that the blepharoplast of *Marsilia* holds the same relation to the poles of the spindles as a centrosome. But Belajeff's conception of the centrosome ('99, p. 204) is that of a morphological and dynamic center which may or may not be easily demonstrated according to the amount of stainable substance present. From these discussions it is

evident that final judgment cannot be passed until certain questions of fact are established by reinvestigations. Shaw and Belajeff cannot both be wholly correct in their observations and interpretations and much depends upon the exactness of future studies upon Marsilia, other pteridophytes, and in the bryophytes. The problems are also related to the processes of zoöspore formation among the thallophytes.

With respect to the bryophytes Ikeno (: 03) has recently published an account of spermatogenesis in *Marchantia polymorpha*. He reports for the mitoses in the antheridium, preliminary to the differentiation of the sperm mother-cells, that a centrosome appears at the side of each nucleus and divides, the two daughter bodies passing to opposite sides of the nucleus and becoming the poles of the spindle. He gives evidence that the daughter centrosomes sometimes divide again when at the poles of the spindle in anaphase. The centrosome cannot be found at the side of the daughter nucleus after the mitosis is completed but it appears when the nucleus is ready for the next division. Ikeno's explanation of the reappearance of the centrosome is unusual. He believes that the centrosome is formed within the interior of each nucleus as a deeply staining body among the linin threads. This body moves to the nuclear membrane and is thrust out into the cytoplasm through a protuberance from the nucleus. It then lies outside of the nucleus and becomes the functioning centrosome, dividing to form two centrosomes that separate to preside over the poles of the spindle. After the final mitoses in the spermatogeneous tissue the centrosomes remain to become the blepharoplasts of the sperms. Each blepharoplast passes to the plasma membrane of its sperm cell and develops two cilia. There is formed at this time another deeply staining body in the cytoplasm considered by Ikeno equivalent to a "Nebenkörper." The nucleus begins to elongate and the "Nebenkörper" takes a position between it and the blepharoplast and in this manner the much attenuated sperm is organized from the mother-cell.

Ikeno considers the blepharoplast of *Marchantia* to be actually a centrosome as shown by its behavior during mitosis. His account therefore in the main supports Belajeff's interpretation

of the blepharoplastoids of Shaw which as just described are regarded by the latter author as centrosomes. Both Belajeff and Ikeno are inclined to use the term centrosome with a looseness that is unusual since the first accounts of this structure gave to it a place in the cell which is not strictly followed in these authors' descriptions of spermatogenesis. Ikeno's account of the intranuclear origin of the centrosome is extraordinary. Intranuclear centrosomes have been reported in several animal forms but they do not leave the nucleus in the manner described by Ikeno.

On the whole the writer is more in sympathy with the views of Webber (:01, pp. 70 to 81), Strasburger and Shaw than those of the other authors. Assuming that the observations upon the cycads and Ginko are correct, Webber is certainly justified in emphasizing the striking fact that the blepharoplasts are completely independent of the spindle in the body cell and that they are formed *de novo* at a distance from its nucleus. These are peculiarities which, if established generally throughout spermatogenesis in plants, will remove the processes entirely from the activities of centrosomes in certain thallophytes (*c. g.* *Stypocaulon*, *Dictyota*) and in many animal cells. It is certainly to be expected that a centrosome when present will always hold an intimate relation to spindle formation during mitosis. It need not be a permanent organ in cell genesis and an ever increasing number of investigations indicate that it frequently is not. Therefore many authors hold that the centrosome is rather the morphological expression of a dynamic center than a protoplasmic structure with an individuality comparable to the organs of a cell. But these universal characteristics of centrosomes are apparently not present in the blepharoplasts of the gymnosperms nor, according to Shaw, in the pteridophytes (*Marsilia*). But then the observations of Belajeff and Ikeno are not in accord with those of Shaw and it is possible that studies in zoöspore formation and gametogenesis among the thallophytes may present the subject in new lights.

For as shown in our discussion of the zoöspore it is not clear whether the blepharoplasts in those cells are always derived in the same manner. We have Strasburger's view that the



structures are thickenings of the outer plasma membrane (hautschicht) and opposed to this Timberlake's account for Hydrodictyon in which the blepharoplast is considered as a structure independent of the plasma membrane although lying in contact with it. It must be apparent that the results of Timberlake are in essential agreement with the events of spermatogenesis in the pteridophytes and gymnosperms while those of Strasburger introduce new elements in giving to the plasma membrane the functions of forming a blepharoplast. The process of spore formation in the ascus must also be considered in this connection for in that sporangium a centrosphere associated with each nucleus develops numerous fibrillæ that resemble so much a cluster of cilia as to suggest at once a blepharoplast-like structure, but this centrosphere of course is an important factor in spindle formation during the mitoses in the ascus. Indeed we may well ask for further studies in spermatogenesis and zoöspore formation before we can expect a solution of the problem of the blepharoplast.

Comparisons have been made between the sperms of animals and plants, and some authors (*e. g.* Wilson :00, p. 175, Belajeff '97c) consider the two cells in essential agreement as to structure and development. However these views rest on the assumption that the blepharoplast is truly the homologue of a centrosome. It seems to be established that the locomotor apparatus of the animal spermatozoön is derived chiefly from one or more centrosomes, generally with the co-operation of archoplasm (idiozome, Nebenkern) present in some form near the nucleus. It is true that in plants the locomotor apparatus is derived from kinoplasm which as we pointed out in Sections I and II corresponds closely to the archoplasm of Boveri, but this is very far from implying that structures formed by the archoplasm and kinoplasm respectively need be homologous. Indeed both archoplasm and kinoplasm are distinguished by their physiological activities rather than by their morphological manifestations which are too various to allow of close genetic relationships. Therefore it seems far from established that spermatogenesis in plants is along the same lines as in animals, especially since the weight of evidence at present indicates that the blepharoplast is not a centrosome.

There are numerous problems connected with the physiology of the sperm that bear directly upon its protoplasmic structure. Some of these will be treated in Section IV in connection with processes of fertilization. But at this time it is well to call attention to the intimate association that sometimes exists between the nucleus and blepharoplast. These structures come into actual contact in *Cycas* and *Ginko* through a process put forth from the nucleus. It should also be remembered that Timberlake and Dangeard found the blepharoplasts in the zoöspores of *Hydrodictyon* and in the cells of *Polytoma* connected with the nucleus by one or two fibers. The nuclear beak that bears the aster in the ascus suggests a similar relationship. These conditions indicate that the activities of locomotion may depend vitally upon the nucleus.

### 3. The Egg.

The subject of fertilization is reserved for the next section (Section IV) of this series and the present account will deal only with the structure of the unfertilized egg. As the sperm is derived from a motile gamete identical with the zoöspore, so the egg has had a similar origin. We have traced the steps in this evolutionary process among the algæ in a former paper (*Popular Science Monthly*, Feb. 1903, p. 300). The first indication of a differentiation in the sex of primitive gametes is one of size. The male gametes tend to become smaller while the female contains a greatly increased amount of cytoplasm. One of the important factors determining this differentiation is the number of nuclear divisions which take place in the cells that produce respectively eggs or sperms. There are generally a great many more mitoses in antheridia than in oögonia and consequently a given amount of protoplasm must be very much divided to provide each nucleus with its quota of cytoplasm.

The tendency of oögenesis on the contrary is to conserve the protoplasm for relatively few nuclei, provided for several eggs or for a single nucleus in a solitary egg, with the result that the egg cell is generally richly supplied with protoplasm. Such processes result in large cells with a prominent chromatophore or

numerous plastids and not infrequently a considerable amount of food material. The primitive female gametes were provided with cilia like the male, but with their increase in size came a sluggishness of movement which resulted in much shorter periods of motility on the part of these sexual cells. There are some algæ (*Ectocarpus siliculosus*, Cutleria, Aphanochæte) whose motile female gametes come to rest shortly after their escape from the oogonia and are fertilized as quiescent cells by the active sperms. These female gametes at the time of fertilization behave physiologically like eggs although their development shows a morphology identical with the sperm. When such female gametes dispense with cilia entirely they become eggs.

The absence of cilia does away with very much of the complexity which we have just described for sperms. There is no trace of the blepharoplast in the egg and no indication of the activities associated with this structure, so conspicuous in spermatogenesis. The large motile female gametes of such Algæ as Bryopsis, Cutleria, Aphanochæte and certain species of Chlamydomonas and Ectocarpus will probably show some interesting conditions when the details of their cell structure and development are known, for some of these types are likely to throw light on the relation which the blepharoplast bears to other structures in the cell.

The eggs of all plants (Fungi excepted) are believed to be richly stocked with plastids in sharp contrast to the sperms which are entirely destitute of these structures in all groups above the algæ. The plastids in the eggs of Algæ contain the pigments characteristic of the respective groups giving these cells a very rich coloration and sometimes an elaborate internal structure since these plastids or the single chromatophore generally maintain a symmetrical relation to the nucleus. Leucoplasts (see Fig. 11a) have been found in the eggs of angiosperms (Schimper, '85) but detailed studies on the cytoplasm of such cells in spermatophytes, pteridophytes and bryophytes are greatly to be desired to determine the history of plastids during the development of these germ cells and at later periods after fertilization.

The distribution of the plastids in the eggs of Algæ may be so general that the entire cell is colored as in *Fucus*, *Volvox* and *Sphæroplea*. Or, the plastids may be largely or wholly withdrawn from some portion of the egg. It is usual for eggs retained within the parent cell (oögonium) to present a colorless area of protoplasm that becomes the point at which the sperm fuses with the egg. Such a hyaline region is called the receptive spot and is generally situated (see Fig. 11*b*) at the side of the egg nearest the pore or opening in the oögonium through which the sperms enter. Excellent illustrations are presented among the Algæ in *Vaucheria* (Oltmanns, '95), *Cedogonium* (Pringsheim, '58, Klebahn, '92) and *Coleochaete* (Pringsheim, '60, Oltmanns, '98). It has been suggested that the receptive spot is related to the clear ciliated end of the ancestral motile gamete and zoöspore but the structures have not been critically compared to determine the precise character of their protoplasmic structure and development. The receptive spot in some forms (*Vaucheria*, *Cedogonium*, Fig. 11*b*) lies directly under the opening that is formed in the oögonium and its protoplasm is probably concerned with the fermentative action that destroys the wall at that point.

The red Algæ (*Rhodophyceæ*) do not have eggs although in their sexual evolution they are at the level of heterogamy. The female gamete (carpogonium with its trichogyne) is a cell homologous with an oögonium and its protoplasmic contents correspond to an egg, but the protoplast never withdraws from the cell wall to lie freely as a naked mass of protoplasm within the structure. But the general agreement of the carpogonium and trichogyne with the oögonium and its neck like extension in *Coleochaete* seems to determine without doubt the homologies of the former.

There are very few eggs among the fungi that are strictly comparable to those of the Algæ. *Monoblepharis* (Thaxter '95*a*) however unquestionably furnishes such an example. But the eggs of the *Saprolegniales* and *Peronosporales* are probably in the author's opinion not directly derived from those of Algæ. They are either a peculiar form of sexual cell called the cœnogamete (Davis :00 and :03) or closely related to this structure

which will be given a separate treatment in this section. The cœnogamete is the homologue of a multinucleate gametangium but its evolutionary tendencies seem to be towards such a reduction in the number of nuclei that in the highest expression of its sexual differentiation the female cell contains a single nucleus and has the general form of an egg. But this process of sexual evolution is entirely independent of the well known lines of development in the Algæ (Davis, *Popular Science Monthly*, Feb. 1903). The female sexual cell of the Ascomycetes (called the ascogonium or archicarp) is probably in most forms the homologue of a gametangium. These subjects will be treated in our account of the cœnogamete.

The egg in the archegonium of bryophytes and pteridophytes is generally reported to have a clearer region on the side nearest the neck and this is called the receptive spot. It is reported by Campbell in his investigations on *Pilularia* ('88), *Isœtes* ('91), *Osmunda* ('92a), *Marsilia* ('92b), and *Marattia* ('94), by Shaw in *Onoclea* ('98) by Thom in *Aspidium* and *Adiantum* ('99) and by Lyon in *Selaginella* (:01). The receptive spot is generally believed to be a portion of the egg differentiated to receive the sperm. It is an open question whether this area is morphologically the homologue of the receptive spot in the eggs of algæ and the clear area at the ciliated end of motile gametes and zoöspores. The problem demands a detailed study of the finer protoplasmic structure to determine whether or not it is kinoplasmic in character. The nucleus is generally situated near the center of the egg and the portions of the cell farthest away from the neck of the archegonium contain coarsely granulate protoplasm which is evidently trophoplasmic, *i. e.*, much of its substance is of the nature of food material and the products of metabolism. The leucoplasts would be supposed to lie in this region of the cell but we know nothing of their presence and behavior in the egg of bryophytes and pteridophytes.

The eggs of gymnosperms generally speaking present sharp contrasts to those of pteridophytes. They are very large, probably the largest uninucleate cells in the plant kingdom, and consequently very attractive for cell studies and some of the best work on the events of the maturation and fertilization of plant

eggs has been done on this group (to be treated in Section IV). Passing over earlier investigations that described accurately the general structure of the egg of gymnosperms we shall consider the results of a number of comparatively recent papers that treat especially the pine, spruce (*Picea*), hemlock (*Tsuga*), fir (*Abies*), cycads, Ginko, Gnetum, *Taxodium*, etc.

Oögenesis and fertilization in the pine has been the subject of several extensive studies the chief being papers by Dixon ('94), Blackman ('98), Chamberlain ('99) and Ferguson (:01b). The protoplasm of the egg is at first vacuolate but later takes on a denser structure which becomes very puzzling because of numer-

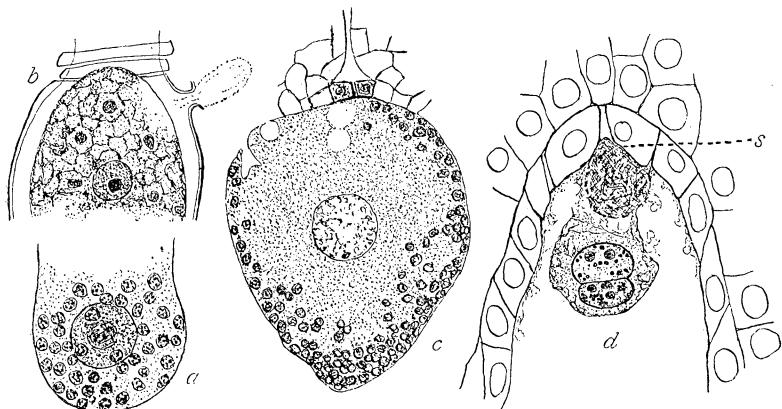


FIG. 11. — The Egg. *a*, *Daphne*, showing leucoplasts; *b*, oögonium, showing receptive spot; *c*, pine, with numerous proteid vacuoles; *d*, embryo sac of the lily, gamete nuclei fusing, remains of one Synergid (*s*) shown. (*a*, after Schimper, '85; *b*, Klebahn, '92; *c*, Ferguson, :01.)

ous granular inclusions and masses of amorphous material which together with fibers present a very complex texture. The fibers are sometimes collected in fascicles and they may form a sort of weft at the periphery of the egg or radiate out from the nucleus which is generally surrounded by a kinoplasmic sheath. The complexity is greatly increased as the egg grows older by the development of remarkable structures called proteid vacuoles (See Fig. 11*c*) which have been especially described by Blackman and Ferguson. The number of proteid vacuoles is exceedingly variable in the egg but they sometimes fill three fourths of the structure. They are spaces in the cytoplasmic reticulum filled

with granules and irregular masses of a proteid nature some of which stain like nucleoli. The proteid vacuolee were considered nuclei by earlier writers (Hofmeister and Goroschankin) and recently this view has been revived by Arnold (:oob) who describes the migration of large numbers of nuclei from the cells of the jacket surrounding the egg into that structure. These results have not been confirmed by Ferguson who agrees with the interpretation of other writers that the resemblance of the proteid vacuoles to nuclei is superficial. Miss Ferguson believes that the material of the proteid vacuoles is derived in part from the nucleoli in the cells of the jacket and from those in the egg. A vacuole is reported (Ferguson) at the end of the egg nearest the neck of the archegonium and this is regarded as a sort of receptive spot since the pollen tube discharges its contents into this cavity. The egg nucleus is very large and its contents are not arranged with the regularity generally present in resting nuclei. There are numerous bodies which Chamberlain believes to be chromatic in composition that look very much like nucleoli and have been so designated by that writer. But there is generally one large unquestioned nucleolus and besides this many smaller nucleoli are reported by Ferguson as held in the linin reticulum. Then portions of the linin frequently take irregular forms and stain heavily. There is also present besides the linin, chromatin and nucleoli much granular material (metaplastm), especially in the nuclei of younger eggs, which probably holds some relation to the chromatin although it may readily be distinguished at certain times from that substance.

Recent accounts of the spruce and fir, by Miyake (:o3a and :o3b) describe conditions very much as in the pine. The egg of the spruce (*Picea*) is apparently not so fibrous in structure but proteid vacuoles give it a coarse granular structure. He finds no evidence in support of Arnoldi's (:oob) peculiar views that the proteid vacuoles are derived from nuclei that have passed into the egg from cells of the sheath. They are simply masses of nutritive material. There is some doubt whether the vacuoles present at the end of the egg really represent a differentiated receptive spot. The egg of the fir (*Abies*) conforms in all essentials to the structure in the pine and spruce. There are numerous proteid vacuoles.

It is probable that the eggs of other conifers will be found to present much the same protoplasmic structure and activities as those of the pine. Thus Murrill (:00) describes for the hemlock spruce (*Tsuga*) a vacuolar receptive spot and figures masses of food material very much like the proteid vacuoles. The general features of the egg of *Cephalotaxus* (Arnoldi, :00a), *Thuja* (Land, :02), *Podocarpus* (Coker, :02), *Taxodium* (Coker, '03) have been recently described and those of *Abies*, *Larix* and *Taxus* are familiar from older writers but the pine remains as the type of conifer in which the events of oögenesis are best known as regards the details of protoplasmic activities.

Besides the pine we have had some very complete investigations on cycads and Ginko (Hirase, '98, Ikeno, '98b and :01, Webber, :01). In some respects these types and especially the cycads seem to be the most favorable of all the gymnosperms for the study of gametes and the processes of fertilization (to be described in Section IV). The cytoplasm of the egg is comparatively homogeneous in structure so that the cell is relieved from the complicated fibrous structure and proteid vacuoles present in the pine. Ikeno ('98b) finds that the egg of *Cycas* develops a crater like depression just before and at the time of the fusion of the sperm thus presenting a rather highly specialized receptive spot.

We know almost nothing of the detailed structure of the egg in the Gnetales. *Ephedra* (Strasburger, '72) develops archegonia much like those of other gymnosperms and we should not expect their eggs to be materially different even in details. But the conditions in *Tumboa* (*Welwitschia*) are peculiar and approach more closely those of angiosperms where the egg nucleus is scarcely differentiated from neighboring nuclei lying freely in the protoplasm at one end of the embryo sac. The eggs of *Tumboa* (Strasburger, '72) are merely cells of the prothallus that push out small projections to meet the pollen tubes. *Gnetum* presents a further simplification or reduction since the female nuclei lie freely in the protoplasm at one end of the embryo sac. In *Gnetum gnemon* the lower half of the embryo sac is filled with a tissue (Lotsy '99) but in several other species studied by Karsten ('92, '93) no cell walls are found in the entire sac until after fertilization.



The angiosperms present no especial advance over Gnetum in the organization of the egg except that this structure is generally reduced to a single female nucleus and the cytoplasm immediately around it (see Fig. 11 *d*). This egg nucleus flanked by two companions (synergids) and the accompanying protoplasm compose the egg apparatus whose morphology is still a matter of dispute. It is possible that the synergids may stand for portions of a reduced archegonium, but the two nuclei bear such close relations to the egg and polar nucleus that it seems very probable that they are homologous with these structures which have clearly defined sexual potentialities. In spite of the numerous studies on embryo sacs in various groups of angiosperms we do not yet know precisely how the cytoplasm becomes gathered around the egg nucleus and the synergids. The spindles that are formed between these nuclei in some types (*e. g.*, *Lilium*) have been supposed to lay down walls by means of cell plates. But there are other forms in which the protoplasm seems to separate along planes of vacuoles without relation to spindle fibers.

(*To be continued.*)